# An Outcrossed Canine Pedigree for Linkage Analysis of Hip Dysplasia

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**Canine hip dysplasia (CHD) is a prevalent, debilitating, polygenic disease characterized by hip subluxation and laxity which results in osteoarthritis. We are developing an informative pedigree for linkage analysis of CHD. The seven greyhound founders had excellent hip conformation with high dorsolateral subluxation scores (percentage of femoral head covered by the dorsal acetabulum in a weight-bearing position) of 66 ± 4% (mean ± SD averaged over both hips) and low hip distraction (laxity) indices of 0.14 ± 0.08. Nine greyhounds bred on site had radiographic evidence of ossification in the capital femoral chondroepiphysis at 7.7 ± 0.9 days of age. At 8 months of age they had a mean distraction index of 0.24 ± 0.08 and dorsolateral subluxation score of 76 ± 1%. Of the four dysplastic Labrador retriever founders, three had mean age at onset of capital femoral chondroepiphyseal ossification of 20 ± 7 days of age *n* = 3) and a mean distraction index of 0.46 ± 0.1 accompanied by hip osteoarthritis. Thirty-four $F_1$s had mean onset of capital femoral ossification (10.7 ± 4.0 days of age) and mean dorsolateral subluxation scores (61 ± 12%) similar to the greyhound founders, but distraction indices (0.42 ± 0.2) more similar to the Labrador retriever founders. One $F_1$ had CHD radiographically but none of 20 $F_1$s had osteoarthritis at necropsy at 10 months of age. These data suggested that maximum passive laxity (as measured by the distraction index) and normal osseous conformation (as indicated by a high dorsolateral subluxation score) were both dominant traits and were controlled by separate quantitative trait loci (QTL). Forty-three backcrosses between $F_1$s with the highest hip laxity and greyhound founders had mean onset of capital femoral ossification at 9.9 ± 2.6 days of age. Of 10 dogs in the backcross generation that have reached 8 months of age, 2 had palpable subluxation without marked CHD radiographically. The mean distraction index of these dogs was 0.36 ± 0.16 and the dorsolateral subluxation score was 65 ± 5%. Although dogs in the backcross generation that were three-quarter greyhound had a broad range of hip laxity, a protective effect of the greyhound QTLs for good osseous conformation has mitigated thus far against subluxation and CHD.**

Human hip dysplasia is referred to as congenital dislocation or developmental dysplasia of the coxofemoral (hip) joint (Salter 1968; Weinstein 1987). The disease involves hip joint laxity or subluxation and abnormal development of the femoral head and acetabulum, called dysplasia (Cardinal and White 1992). Delayed mineralization of the affected capital femoral epiphysis has been reported in humans with developmental dysplasia of the hip (Wilkinson 1985). If the human disease goes undetected, is not apparent at birth, or treatment is unsuccessful, hip osteoarthritis ensues (Weinstein 1992). Persistent hip joint pain as a result of osteoarthritis often requires medication or total hip replacement. There is a familial basis for human hip dysplasia that is influenced by environmental factors (Wynne-Davies 1970), but the genetic cause of the human disease remains unknown. Identification of genetic markers and the genes that cause hip dysplasia would facilitate the development of treatments aimed at its cause, aid in early detection and prevention through genetic screening programs, and further the understanding of the genetic basis of complex or quantitative orthopedic traits.

Canine hip dysplasia (CHD) is a prevalent, debilitating, developmental disease also characterized by delayed onset of capital femoral ossification (Madsen et al. 1991; Todhunter et al. 1997), hip joint laxity (Lust et al. 1993; Smith et al. 1990, 1993), and incongruity between the acetabulum and femoral head resulting in subluxation (Lust 1997). The subluxation

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results in joint pain and leads inevitably to a mechanically induced osteoarthritis of the affected hip. Therefore it is a natural model of human hip dysplasia and osteoarthritis (Burton-Wurster et al. 1993; Henricson et al. 1966; Riser 1975). Despite attempts to control CHD through breeding programs, it remains a major orthopedic disease of dogs (Corley et al. 1992; Swensen 1997). The pathogenesis of CHD has been extensively studied (Burton-Wurster et al. 1993; Henricson et al. 1966; Riser 1975), but its underlying etiology and genetic basis are unknown.

Canine hip dysplasia is a quantitative (polygenic) trait (Willis 1989). Based on genetic linkage analysis of quantitative traits in other animal species and plants, it is likely that a few major quantitative trait loci (QTL) with minor contributions from many others, produce the phenotype of CHD (Lander and Botstein 1986; 1989; Paterson et al. 1988; Tanksley 1993). Like many quantitative traits, variation in the CHD phenotype is further determined by environmental factors such as food consumption; decreased food consumption significantly decreased the severity of hip osteoarthritis in dogs affected with CHD (Kealy et al. 1992, 1997).

Clinical and radiographic methods are used for diagnosis of CHD at maturity, but a normal dog may carry alleles for CHD (Kaneene et al. 1997). With a genetic marker for the early detection of CHD-susceptible dogs, we could test for disease susceptibility before breeding age and potentially intervene with optimal therapy before the development of osteoarthritis. Identification of genetic markers and mutations in other pedigrees should follow because the linked markers that are identified in some pedigrees may not be broadly applicable to the dog population and different mutations may be segregating in different pedigrees.

We can optimize the chance of detecting linkage to genes causing CHD by developing pedigrees in which different alleles are segregating among dogs with low and high susceptibility to CHD. Our objectives are to find molecular genetic markers that are linked to the QTLs that cause the variation in CHD phenotype and the mutations that cause the disease. We describe the development and phenotypic characteristics of an outcrossed pedigree for linkage analysis of CHD.

## Materials and Methods

Our strategy has been to establish genetic linkage to CHD based on objective measurements of the phenotype, canine microsatellite markers, and an informative pedigree.

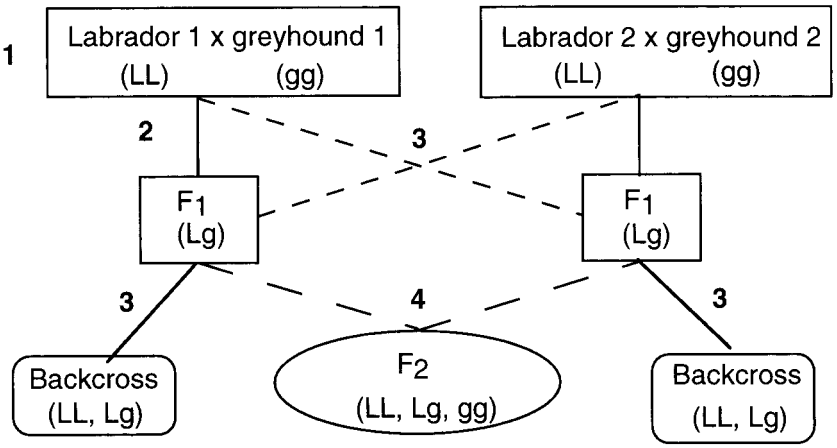


**Figure 1.** Scheme illustrating the construction of an outcrossed Labrador retriever–greyhound pedigree for genetic linkage analysis of CHD. 1. Founding greyhounds had excellent hip conformation and are presumptively homozygous normal at the QTLs for CHD and the founding Labrador retrievers are presumptively homozygous for the mutant alleles at the QTL for CHD. 2. Crossbreeding them produces the heterozygous $F_1$ generation. At this level we can determine statistically whether hip joint laxity as measured by the distraction index, subluxation as measured by the dorsolateral subluxation score, and capital femoral ossification are dominant or recessive traits. 3. Backcrossing $F_1$s affected with hip laxity to the greyhound parents of other litters should result in cosegregation of microsatellite markers with the laxity and dorsolateral subluxation trait. Backcrossing $F_1$s to Labrador retriever parents of other litters should result in cosegregation of markers with the subluxation trait. 4. Genotyping and phenotyping each extreme of the $F_2$ generation ($F_1 \times F_1$) would maximize the chance to find linkage. Linkage is established when a proportion of the phenotypic variation can be explained by the presence of a particular microsatellite at a statistically significant level. Alternatively, for a dichotomous variable, like the presence or absence of osteoarthritis, as a phenotypic marker of CHD, linkage is present when the log of the odds ratio (LOD score) is greater than 3 (a recombination frequency of less than 1:1000 meioses).

### Informative Pedigree

Quantitative trait mapping relies on linkage maps based on DNA markers (Andersson et al. 1994; Daniels et al. 1996; Edwards et al. 1991; Falconer 1993; Holmes 1994; Lander and Botstein 1986, 1989; Paterson et al. 1988; Ron et al. 1994; Tanksley 1993; Weber and May 1989). These genetic markers must be reproducible and the pedigree on which they are used should be derived from founders that express normal and affected phenotypic characteristics (Lui 1998). An outcrossed pedigree was founded with greyhounds that have excellent hips and Labrador retrievers that have dysplastic hips. Because of inbreeding and selection, we assumed that the greyhounds were homozygous normal at the QTL for CHD and the affected Labrador retrievers were homozygous for the mutation at each major QTL (Figure 1).

The dysplastic Labrador retrievers were likely to be homozygous for the mutations at the major QTL that cause the dysplastic phenotype because these dogs have been used to study the pathogenesis of CHD (for a recent review see Lust 1997) and osteoarthritis (for review see Burton-Wurster et al. 1993) for the last 29 years in an inbred colony, not designed to optimize genetic linkage analysis. Further, because of the high incidence of CHD in progeny from phenotypically normal Labrador retrievers [about 25% (Willis 1989)], our phenotypically normal Labrador retrievers must carry some alleles for CHD. In contrast, greyhounds have been selected for racing, have a low incidence of CHD and low hip laxity, and are unlikely to carry disease mutations.

Crossbreeding Labrador retrievers and greyhounds, with and without CHD, respectively, followed by backcrossing $F_1$s to the greyhounds and Labrador retrievers and intercrossing the $F_1$s would maximize our chances of establishing a broad phenotypic range from normal to the worst dysplastic hips (Figure 1). The greater the phenotypic difference between two individuals, the more likely the detection of QTL that control that character in a derived, segregating population (Tanksley 1993). Controlled matings result in maximum linkage disequilibrium for detecting QTLs with linked molecular markers (Tanksley 1993). Backcrossing to the greyhound would recover the recessive phenotype with regard to laxity, and to the Labrador retriever with regard to subluxation (CHD). This would enable us to discriminate the backcross phenotypes based on presumptive homozygous recessives (low laxity and dysplastic for back-

**Table 1. Hip joint laxity (distraction index) of founding Labrador retrievers and greyhounds**

| Labrador retrievers (D)[a] | | | Greyhounds (N)[b] | | |
|---|---|---|---|---|---|
| Dog | Gender | Distraction index[c] | Dog | Gender | Distraction index[c] |
| B53 | Male | 0.52 | 381 | Female | 0.15 |
| Baron | Male | 0.42 | 387 | Female | 0.10 |
| B45 | Female | 0.66 | 380 | Male | 0.10 |
| A15 | Male | 0.58 | 388 | Male | 0.06 |
| A14[d] | Female | 0.35 | 385 | Female | 0.07 |
| Andy | Male | — | 222 | Female | 0.16 |
| C 45 | Female | 0.9 | 223 | Female | 0.04 |

[a] Labrador retrievers distraction index at 8 months of age. In older dogs, when disease was more advanced, distraction index was not useful (capsular hypertrophy interferes with laxity). Therefore, there is no distraction index for Andy.

[b] Greyhounds were between 2 and 3 years old at the time of radiography. D = dysplastic on standard radiographic examination; N = normal (no abnormalities) on standard, hips-extended examination.

[c] Distraction index is the ratio of the displacement (distance in millimeters) between the geometrical centers of the femoral head and acetabulum divided by the radius of the femoral head (in millimeters). The distraction index listed is averaged over each hip per dog.

[d] This dog was phenotypically normal (moderate hip quality) at 2 years of age.

crosses to greyhound and Labrador retriever, respectively) and the heterozygotes (lax and nondysplastic, respectively).

***Dogs and breedings.*** Greyhounds and dysplastic Labrador retrievers were bred to establish the informative pedigree. Seven greyhounds (two males and five females) with very good to excellent hip conformation (Table 1) were purchased from racing stock. The four dysplastic Labrador retrievers (Table 1) were selected from our colony that includes both phenotypically dysplastic and normal (nondysplastic) breeders. About 85% of our dysplastic dogs are affected in both hips (Todhunter et al. 1997). Dogs were bred by artificial insemination based on rising serum progesterone concentrations. Dogs were inseminated between days 3 and 6 after their serum progesterone concentrations reached 1.0 ng/ml.

Feeding regimens were designed to achieve maximum growth rate for maximum expression of CHD (Kealy et al. 1993, 1997). Pregnant and postwhelping bitches were fed a standard diet based on canned (Hill's PD, Hill's Pet Foods Inc., Topeka KS) and ad libitum dry (Big Red High Energy, Agway, Ithaca, NY) food. Pups were introduced to rice cereal, canned, and dry food at 4 weeks of age and were weaned at 6 weeks of age. The study was approved by the Institutional Animal Care and Use Committee.

## Phenotypic Characteristics

Our strategy was to reduce the phenotype into separate components related to joint capsular integrity and osseous conformation. These are the two major structural features (along with muscular support) that stabilize a joint when it is loaded and muscles contract across the joint. The neonatal age at onset of ossification in the secondary center of the capital femoral epiphysis was used as an early phenotypic measure of disease susceptibility (Todhunter et al. 1997). The standard, hip-extended [Orthopedic Foundation for Animals (OFA)] method was used to assess hip subluxation and the presence of osteoarthritis (Henry 1992). The distraction index (PennHip™, Malvern, PA) method (Smith 1997; Smith et al. 1993) was used to measure maximum lateral passive laxity of the hip. The dorsolateral subluxation (DLS) test was used to measure the amount of passive dorsolateral subluxation of the hip in a weight-bearing position (Farese et al. 1998). Dogs were palpated for hip subluxation by the Ortolani maneuver (Chalman and Butler 1985). For this maneuver, the dog is placed in lateral or dorsal recumbency with the femur perpendicular to the spine and the stifle held at 90 degree flexion. A dorsal force is placed on the femur through a cupped hand on the stifle to elicit a dorsal and lateral subluxation of the femoral head. While continuing to apply the load, the stifle is abducted and the reduction of the luxation is felt as a click or clunk (a positive Ortolani sign).

***Onset of femoral head ossification.*** The pelvis of each pup was radiographed in the hip-extended position every other day from 6 days of age until the secondary center of ossification in the capital femoral epiphysis was observed. Previous studies showed that dysplastic German shepherds (Madsen et al. 1991) and Labrador retrievers (Todhunter et al. 1997) had significantly later onset of femoral head ossification (mean ± SD; 17.1 ± 6.3 days for Labrador retrievers) than their nondysplastic breed counterparts (13.8 ± 1.7 days).

***Hip-extended, radiographic view.*** The hip-extended radiographic projection was obtained at 8–12 months of age (and repeated later if the dogs remained in the colony) or at maturity for the greyhound founders that were purchased as adults. Labrador retrievers and greyhounds were premedicated with acetylpromazine (0.02 mg/kg, intramuscularly) and glycopyrrolate (0.01 mg/kg, intramuscularly); general anesthesia was induced with intravenous thiopental (6–10 mg/kg intravenously; Veterinary Pentothal, Abbott Laboratories, North Chicago, IL) and maintained with halothane (Halocarbon Laboratories, River Edge, NJ) after intubation. For greyhounds, general anesthesia was induced with propofol (6 mg/kg, intravenously, Diprivan, Zeneca Pharmaceuticals, Wilmington, DE) prior to intubation and inhalation anesthesia (Robertson 1992; Zoran et al. 1993). Greyhounds do not redistribute barbituates well due to high muscle:fat ratios, so other induction agents are necessary to avoid prolonged anesthesia (Sams et al. 1985). For subsequent generations, dogs that were 50% or more Labrador were induced with thiopental and others with propofol.

The dogs were positioned in dorsal (supine) recumbency with the hips extended so that the femora were parallel to the tabletop (Rendano and Ryan 1987). A ventrodorsal (anteroposterior) radiographic projection was taken with the dogs in this position (Figure 2A,B). The projections were scored independently for the presence or absence of CHD (subluxation) and osteoarthritis based on the 7-point scale adopted by the OFA (Henry 1992).

***Distraction index.*** The maximum amount of lateral hip laxity was measured on a distraction radiographic projection. The measurement is called the distraction index (PennHip™) (Smith 1997; Smith et al. 1993). For the distraction radiograph, the dogs were anesthetized and placed in dorsal recumbency. The hind legs were held vertical to the X-ray table (neutral position) and a distractor was fixed between the dog's thighs and secured firmly to the table with velcro straps. By adducting the dog's stifles, the femoral heads were distracted from the acetabulae by the fulcrum effect of the distractor over the dog's hip joints, and a radiograph was taken (Figure 2C,D). Circular gauges with known diameters were placed separately over each femoral head and acetabulum on the radiograph and the distance between the center of each circle was measured for each joint. The distraction index was calculated by dividing this distance by the radius of the femoral head to give a number which ranged from 0 to 1 (Smith et al. 1993). Labrador retrievers with distraction indices less than 0.3 at 8 months of age have a high probability of normal hips (no osteoarthritis). Labrador retrievers with distraction indices greater than 0.7 have a high probability of developing osteoarthritis and CHD (Lust et al. 1993).

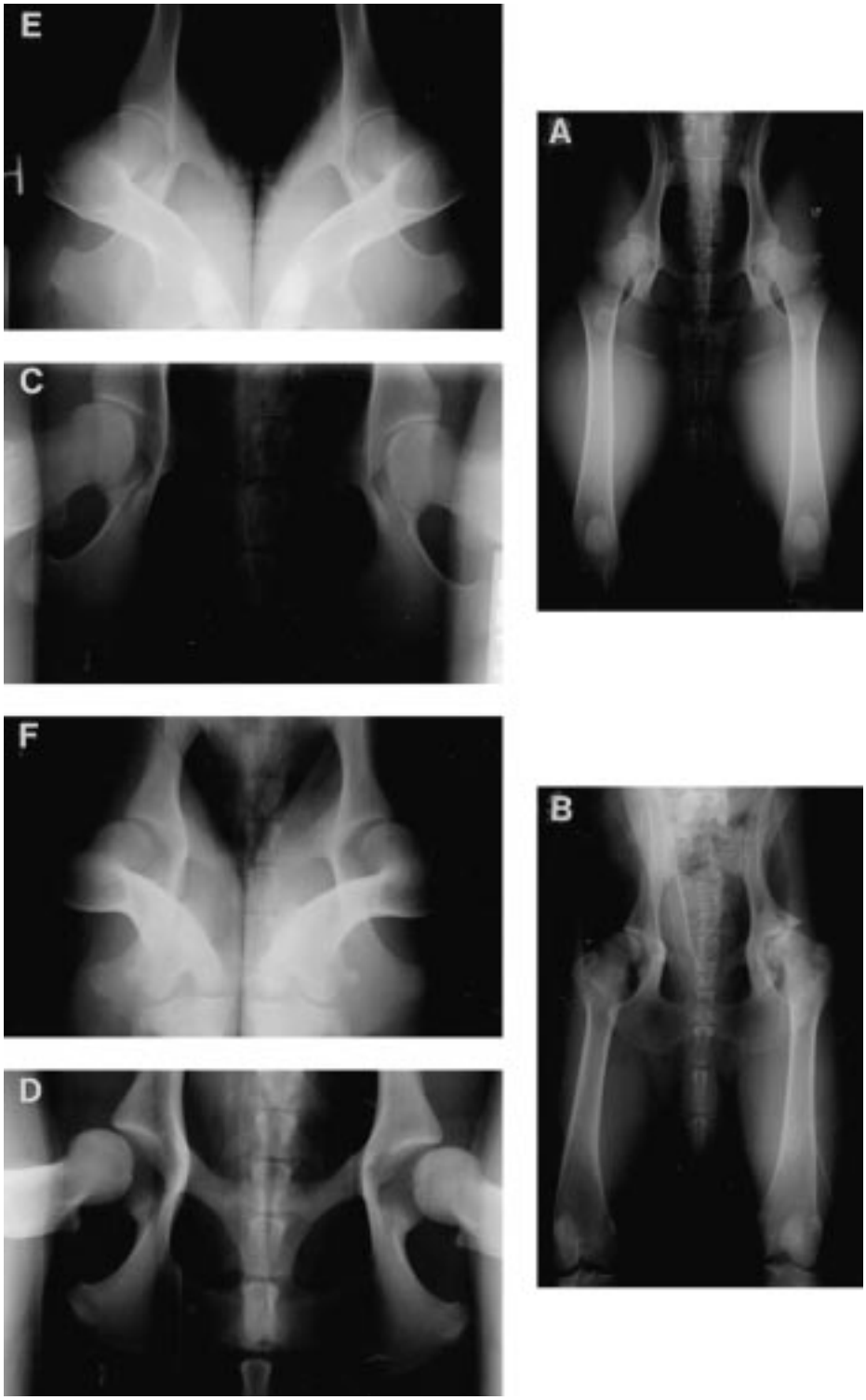


**Figure 2.** Photographs of pelvic radiographs of a dysplastic Labrador retriever and a greyhound. **(A, B)** Pelvic radiograph taken in the supine position with the hind limbs fully extended (Orthopedic Foundation for Animal's position). The femoral heads of the greyhound in (A) are congruent with the acetabulum (cup), but in (B) the femoral heads are subluxated in this dysplastic Labrador retriever. Osteoarthritis is evident in 2B as a flattening (remodeling) of the femoral head and osteophytes and enthesiophytes (lipping) at the acetabular margin. **(C, D)** Photographs of radiographs taken during the PennHip™ procedure to measure maximum passive hip joint laxity (distraction index). Note the excellent congruence of the hip joint of the greyhound (C) resulting in a very low distraction index and the lateral displacement of the hips of the Labrador retriever (D) resulting in a high distraction index [0.6 for left hip, 0.64 for right hip (on viewer's left)]. The shadow of the distractor that is used as a fulcrum can be seen superimposed over each hip. See Materials and Methods for explanation of the technique for measurement of the distraction index. **(E, F)** Photographs of dorsoventral (posteroanterior) pelvic radiographic projections of dogs in the dorsolateral subluxation (weight-bearing) test position for measurement of the dorsolateral subluxation score. Note the good congruity of the femoral heads and acetabulae of the greyhound in (E) (percent femoral head coverage or dorsolateral subluxation score was 63% for the left hip and 67% for the right hip) and the dorsolateral subluxation of the femoral heads of the dysplastic Labrador retriever in (F) (dorsolateral subluxation score was 44% for the left hip and 41% for the right hip 40%).

***Dorsolateral subluxation score.*** The dorsolateral subluxation (DLS) test is a radiographic method of eliciting dorsolateral subluxation of the femoral head from the acetabulum with the hips in a weight-bearing position (akin to functional laxity) (Farese et al. 1998). To determine the DLS, anesthetized dogs were placed in sternal (prone) recumbency; stifles were flexed and placed in a kneeling position within a hole cut in a foam-rubber pad. Natural hind limb weight-bearing forces were transmitted to the hips by flexing and adducting the stifles with the femora perpendicular to and in contact with the tabletop. The DLS score was measured as the percentage of femoral head covered by the craniodorsal acetabulum on a dorsoventral (posteroanterior) radiographic projection (Farese et al. 1998) (Figure 2E,F).

***Environment.*** To test local environmental factors that might influence phenotypic expression of CHD in the pedigree, we bred a greyhound × greyhound litter. These dogs were radiographed as described. The phenotypic measurements for these dogs also served as a best estimate of the phenotype of the founding greyhounds when they were 8 months of age.

***Necropsy.*** The definitive diagnosis of CHD was based on the presence of subluxation or hip osteoarthritis on a radiograph or on the presence of osteoarthritis at necropsy (Lust 1997). All crossbreeds not retained for breeding and not adopted were euthanized at maturity (older than 8 months of age) with an overdose of pentobarbital sodium. Their hip joints were evaluated for the presence or absence of osteoarthritis, including synovial effusion and hypertrophy of the teres ligament (Lust and Summers 1981). The earliest osteoarthritic cartilage lesions include fibrillation in the dorsal perifoveal femoral head extending to cartilage erosion and ulceration with destruction of the teres ligament.

***Selection of $F_1$s.*** Dogs in the $F_1$ generation with the highest distraction indices and of both genders were retained in the colony and were bred to greyhound and Labrador retriever parents (founders) of other $F_1$ litters and intercrossed to produce an $F_2$ generation (Figure 1).

## Results

### Pedigree

The current status of the outcrossed greyhound–Labrador retriever pedigree for ge-

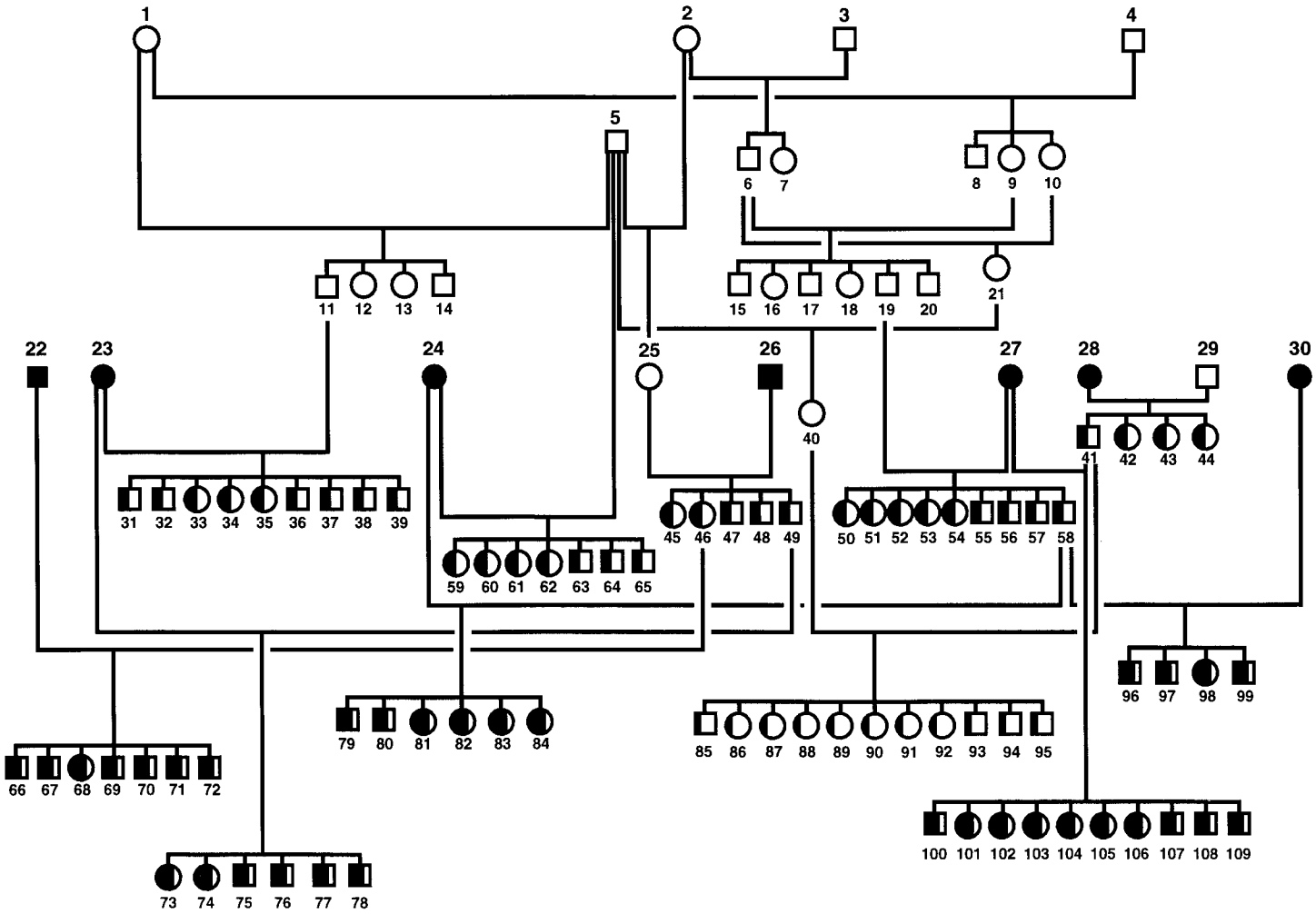


**Figure 3.** Outcrossed (greyhound–Labrador retriever) pedigree. For simplicity, dog identification is by numeric order.

netic linkage analysis of CHD is illustrated in Figure 3. In this figure, the antecedent Labrador retriever lineage has been superimposed on the outcrossed pedigree. Similar information is not available for the greyhound founders. There were seven founding greyhounds and five founding Labrador retrievers whose distraction indices are shown in Table 1. Thirty-four of 37 dogs in the $F_1$ generation survived to maturity. Ten $F_1$s with the highest distraction indices, including one dog with mild radiographic CHD and palpable hip subluxation (positive Ortolani test), were retained for backcrossing and intercrossing (Figure 3). We retained one $F_1$ male with the tightest hips (average distraction index for both hips was 0.11) for backcrossing to a greyhound to test for homozygosity at the major QTLs for hip laxity. Four $F_1$s were adopted and the remaining $F_1$s were necropsied between 8 and 11 months of age.

Six backcross litters (44 dogs) have been produced (Figure 3). The oldest litter is 9 months old and the youngest is 2 months. One of the backcrosses in the first litter of four dogs had borderline-quality hips based on OFA assessment, with a right distraction index of 0.61 and a left DLS score of 55%. This dog had a positive Ortolani sign on the right hip. One dog in the second backcross litter of six had a right distraction index of 0.7 with a right DLS score of 64%.

## Phenotype

***Necropsy.*** Twenty $F_1$s and the six backcrosses necropsied had normal hips.

***Onset of capital femoral ossification.*** Because this method was introduced after some of the founders were born and the greyhounds were acquired as adults, ossification data were not available on all founders. Therefore the age at onset of capital femoral ossification for the greyhound founders was estimated from the greyhound × greyhound litter bred on site (see below). Data was available on four dysplastic Labrador retriever founders that had radiographic evidence of secondary centers of ossification in their capital femoral chondroepiphyses at a mean age of 20 ± 8 days when averaged over both hips (Figure 4). For 34 $F_1$s, these secondary centers were radiographically evident at a mean age of 10.7 ± 4.7 days (Figure 4) and the distribution was skewed to the left (earlier age of ossification). Data thus far suggests that the greyhound effect on

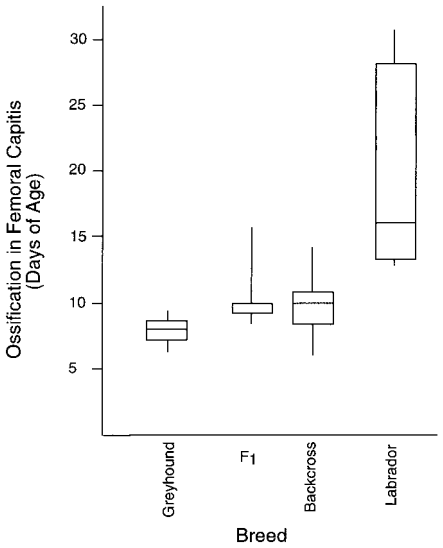


**Figure 4.** Box-and-whiskers plots comparing the distribution of ossification onset in the capital femoral chondroepiphysis between dysplastic Labrador retriever founders ($n$ = 4), a greyhound litter born on site ($n$ = 9), the $F_1$ generation of greyhound × Labrador retriever (founder) breedings ($n$ = 33), and the backcross generation from greyhound founder × $F_1$ breedings ($n$ = 44). The age at onset of the capital femoral chondroepiphyseal ossification of the greyhound litter is the best estimate for the greyhound founders for whom that information was not available. Box-and-whiskers plots show the median, the 25th, and the 75th quartile, and the lowest and highest phenotypic measurement.

onset of ossification was dominant. For 44 backcrosses, the mean age at radiographic ossification was 9.9 ± 2.7 days (Figure 4).

***OFA scores.*** The founding greyhounds had very good or excellent hip conformation (Figure 2A) and the founding Labrador retrievers had dysplastic hips with OFA scores of moderate to severe CHD (Figure 2B). The exception was A14 (Table 1), with borderline hip quality during development but with adequate quality at maturity. This dog may show that there are CHD alleles in phenotypically normal dogs. The $F_1$ generation had OFA scores indicating very good hip quality, except one dog that showed evidence of CHD (subluxation). This dog also had clinical evidence of subluxation (a positive Ortolani sign). Two dogs in the first 10 dogs in the backcross generation at 8 months of age had positive Ortolani signs and 1 had borderline hip quality.

***Distraction index.*** Distraction indices were not available on all founders at 8 months of age because the method was introduced after some of the founders were older, and one founder had osteoarthritis which made the method meaningless due to capsular fibrosis. The mean distraction index of the $F_1$ generation was similar to the mean distraction index of the Labrador retriever founders (Table 2, Figure 5). The exception was the GX96 litter (Table 2) which had a mean distraction index intermediate between both parents. With the addition of two more litters with a dysplastic Labrador retriever female parent, we will analyze the distraction indices of the $F_1$ generation for maternal inheritance and test putative dominance of the laxity and other measurements statistically. The mean distraction index of the first ten 8-month-old backcrosses (to greyhounds) (0.36 ± 0.16) was similar to the mean distraction indices of the $F_1$ generation (0.39 ± 0.1). The distribution of distraction indices for the $F_1$ generation was skewed to the right (higher laxity).

**Table 2. Distraction indices (DI) of the $F_1$ generation at 8 months of age**

| Litter ($n$)[a] | Mean DI (± SD) | Range | Mean DI of parents |
|---|---|---|---|
| GX95 (9) | 0.48 ± 0.1 | 0.25–0.66 | 0.34 |
| AX96 (5) | 0.35 ± 0.07 | 0.26–0.48 | 0.21 |
| BX96 (7) | 0.48 ± 0.14 | 0.19–0.76 | 0.25 |
| FX96 (4) | 0.42 ± 0.09 | 0.20–0.50 | —[b] |
| GX96 (9) | 0.28 ± 0.1 | 0.07–0.42 | 0.31 |

[a] $n$ = number of dogs in the litter.

[b] Labrador retriever parent had too much osteoarthritis for accurate DI.

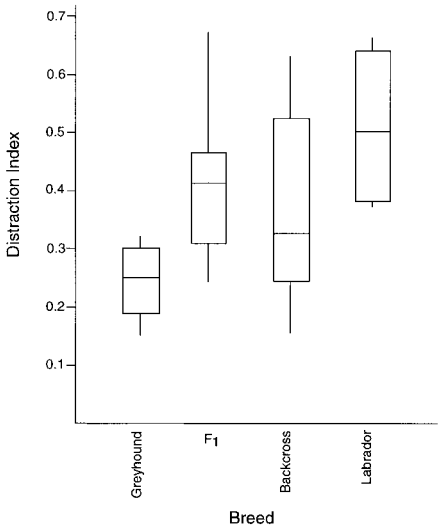


**Figure 5.** Box-and-whiskers plots comparing the distribution of distraction index between dysplastic Labrador retriever founders ($n$ = 4), an estimate for the greyhound founders, a greyhound litter born on site ($n$ = 9), the $F_1$ generation of greyhound × Labrador retriever (founder) breedings ($n$ = 33), and the backcross generation from greyhound founder × $F_1$ breedings ($n$ = 10). The distraction indices of the greyhound litter are the best estimate for the greyhound founders for whom the data at 8 months of age was not available. Data on ten 8-month-old dogs is available thus far for the backcross generation. The remainder of the backcross dogs are less than 8 months old. Box-and-whiskers plots show the median, the 25th, and the 75th quartile, and the lowest and highest phenotypic measurement.

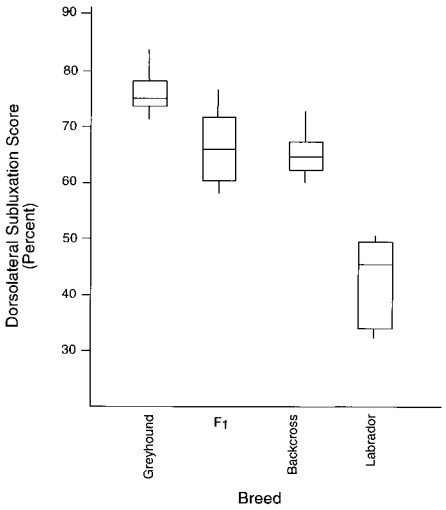


**Figure 6.** Box-and-whiskers plots comparing the distribution of dorsolateral subluxation scores at 8 months of age of dysplastic Labrador retrievers ($n$ = 9), a greyhound litter born on site ($n$ = 9), the $F_1$ generation of greyhound × Labrador retriever (founder) breedings ($n$ = 33), and the backcross generation from greyhound founder × $F_1$ breedings ($n$ = 10). The dorsolateral subluxation scores for the dysplastic, 8-month-old Labrador retrievers are the best estimate for the dysplastic Labrador retriever founders for whom the dorsolateral subluxation scores at 8 months of age were not available. Hip dysplasia in these 8-month-old dysplastic Labrador retrievers was confirmed at necropsy by the presence of osteoarthritis. Thus far, dorsolateral subluxation scores of 8-month-old backcross dogs are available on 10 dogs only. The remainder of the backcross dogs are less than 8 months old. Box-and-whiskers plots show the median, the 25th, and the 75th quartile, and the lowest and highest phenotypic measurement.

***Dorsolateral subluxation scores.*** Data was not available on DLS scores of dysplastic Labrador retriever founders and greyhound founders at 8 months of age. The mean DLS scores for the greyhound founders between 2 and 5 years of age was 66 ± 3%. The estimated mean DLS score for the dysplastic Labrador retriever founders was 42 ± 8%, for the $F_1$ generation was 61 ± 12%, and for the first 10 backcrosses (to greyhounds) at 8 months of age was 65 ± 5% (Figure 6). The estimate for the dysplastic Labrador retriever founders was based on the DLS scores of 11 confirmed dysplastic (osteoarthritis at necropsy), 8-month-old Labrador retrievers (Figure 6). Data suggested that the greyhound effect of good osseous conformation was dominant.

## Environment

The greyhound × greyhound breeding produced nine pups whose mean age at onset of femoral head ossification was 7.7 ± 0.9 days (Figure 4). Their mean distraction index was 0.24 ± 0.08 at 8 months of

age (Figure 5). The highest index for an individual hip was 0.36 and the lowest was 0.11. The distraction index averaged over both hips for the greyhound parents of this litter when they were more than 2 years of age was 0.2. The mean greyhound distraction index at 8 months of age was about half the mean distraction index of the $F_1$ Labrador retriever/greyhound crossbreeds at the same age (Table 2, Figure 5). For the greyhound litter, the mean DLS score at 8 months of age was 76 ± 3% (Figure 6) and the OFA scores were excellent.

## Discussion

Canine hip dysplasia was first described in North America in the 1930s (Schnelle 1935), yet its genetic cause remains unknown. Its polygenic nature has contributed to the difficulty of its eradication. The canine disease phenotype, as in humans, is marked by hip joint laxity and subluxation, but capturing both of these components in a single radiograph and measuring with high sensitivity and specificity the phenotype of CHD has proved difficult. Hence our strategy of reducing the phenotype into subcomponents. The standard diagnostic method for CHD is based on a ventrodorsal, hip-extended radiograph at 2 years of age (OFA view) (Henry 1992). However, Willis (1989) concluded that 64–81% of the progeny of normal parents, based on this radiographic criteria, were normal and 19–36% were dysplastic. The strategy for markedly reducing the incidence of CHD based solely on standard, hip-extended, pelvic radiography has been unsuccessful partly because carriers go undetected and subluxation can be masked by hip extension in the OFA view (Farese et al. 1998). However, when stringent criteria based on this method are applied to determine suitable dogs for breeding in closed colonies (Leighton 1997) or when national hip-screening schemes are compulsory (Swensen 1997), progress can be made in improving hip quality.

In the 1980s, the distraction index for measuring hip joint laxity radiographically was introduced (Smith et al. 1990, 1993). The breeding of dogs with low laxity should reduce the incidence of CHD and the severity of its associated osteoarthritis in breeding colonies. Many dogs have intermediate distraction indices (in the range 0.4–0.7) and one cannot use the distraction index in this range to predict accurately whether such dogs have CHD or will develop osteoarthritis. Because dorsolateral subluxation during loading of the hip results in the development of osteoarthritis, the dorsolateral subluxation score may prove to be a good phenotypic measure of CHD. Until this method is validated, the presence of osteoarthritis on radiographs at 2 years of age or at necropsy remains the gold standard of CHD diagnosis. Although we used different general anesthetic induction agents, all dogs were placed on the inhalant anesthetic halothane for maintenance of general anesthesia. All dogs were at the same depth of anesthesia when the distraction and dorsolateral subluxation test radiographic views were taken.

Because currently accepted radiographic methods for the early diagnosis of CHD are inaccurate predictors of whether an individual dog has CHD or will develop osteoarthritis, a molecular genetic marker for CHD would be very useful for detecting CHD-susceptible dogs as neonates and prior to purchase. With recent advances in QTL mapping (Lander and Schork 1994; Risch and Merikangas 1996), and by example from approaches taken to map quantitative traits in other species (Andersson et al. 1994; Daniels et al. 1996; Holmes 1994; Ron et al. 1994), the time was ripe to attempt QTL mapping of CHD. The first step in QTL mapping was to establish an informative, outcrossed pedigree. Greyhounds were chosen as the prototypical "nondysplastic" dog because they have very low distraction indices with excellent hip conformation (Cardinet et al. 1983; Gustafsson et al. 1975; PennHip™ registry, Malvern, PA). Racing greyhounds have been bred to perform strenuous exercise and have been subjected to strong selection pressure. They are unlikely to carry the alleles for CHD.

Our nondysplastic Labrador retrievers were clearly unsuitable as the founding normal stock. Nondysplastic Labrador retrievers may carry some of the alleles for CHD but not all those required for phenotypic expression of the disease. Use of such dogs as founders would introduce error into the linkage analysis. The broadest phenotypic range of a trait like hip laxity or subluxation would be desirable in the progeny because it maximizes the chance to discriminate phenotypes for linkage analysis. Selection of normal Labrador retrievers as founders would not achieve the low distraction indices characteristic of greyhound hips. The founding dysplastic Labrador retrievers have the loose-hipped phenotype and show hip osteoarthritis radiographically. These dogs were mature before the DLS test was developed as a phenotypic measure of CHD. However, their mean DLS scores, estimated from other dysplastic Labrador retrievers bred in the colony, was low. By outcrossing dysplastic Labrador retrievers with greyhounds, we anticipated that the greatest range in phenotype in the backcross, or $F_2$, progeny would be achieved.

The $F_1$ generation had a body type intermediate between a greyhound and Labrador retriever. They were well muscled and thin skinned. The $F_1$ generation had a greyhound-like phenotype with regard to osseous hip structure, early age at onset of ossification in the secondary center of the capital femoral chondroepiphysis, and high DLS scores. These results concur with the phenotype of German shepherd–greyhound crossbreeds reported by Gustafsson et al. (1974). The mean distraction index of the $F_1$s we bred was similar to the Labrador retriever founders and the distribution was skewed to the right (higher distraction index and toward that of the Labrador retriever). This suggests that the distraction index and DLS score, although significantly correlated when dogs with each extreme of the laxity phenotype were analyzed (Farese et al. 1998), are measuring different components of the dysplastic trait in this outcrossed pedigree. This may partly explain why all but one of the $F_1$s had no subluxation or osteoarthritis but yet had moderate hip laxity. The data suggest that the osseous structure of the greyhound is dominant and "protective" against osteoarthritis (i.e., subluxation and CHD) in the $F_1$ generation even when hip laxity is dominant. Resolving CHD into simpler phenotypes of joint laxity and dorsolateral subluxation may increase the opportunity to find linkage because these phenotypes may be controlled by fewer or even single genes. The measurement of several independent characteristics of the phenotype has precedence in the molecular genetic analysis of other quantitative traits (Andersson et al. 1994; Daniels et al. 1996; Ron et al. 1994).

Yet dorsolateral hip subluxation cannot occur in the absence of passive laxity of the hip, and because laxity is clearly a component of the dysplastic phenotype, finding a genetic marker for hip laxity is important. Laxity is a susceptibility factor for CHD (Smith 1997). In addition, the heritability of CHD as defined by the standard hip-extended radiograph has been reported to range from 0.25 to 0.40 (Hedhammar 1979; Willis 1989), while the heritability of

the distraction index is higher at 0.48 (Leighton 1997). Heritability of the DLS score is, as yet, unknown. The heritability of CHD and hip laxity in our outcrossed pedigree is unknown at present, but should be at least as high as those reported because our colony is maintained in a controlled environment. A controlled environment should reduce the nongenetic component of variance of the CHD phenotype.

Once the founders and $F_1$s were in place, the next step was deciding how to expand the pedigree longitudinally to optimize linkage analysis. According to Darvasi (1997), when one is considering an $F_2$ versus a backcross strategy for QTL detection, an $F_2$ will give a "general picture" of the number of QTLs segregating and estimates of their additive and dominance effects. However, a backcross should be the most efficient for detection of at least some of the major QTLs. For additive effects, an $F_2$ requires about 30% fewer progeny, but to detect dominance a backcross requires about half the progeny of an $F_2$ generation. Based on the assumption that the greyhound and Labrador retriever founders were homozygous at the major QTLs for CHD, the $F_1$ progeny would be heterozygous at these loci. We adopted the approach that dogs in the $F_1$ generation were "generic" in so far as they were all putatively heterozygous at the loci controlling the laxity and CHD phenotype.

In order to find linkage between a trait and a marker that cosegregated with the QTL, breeding back to the greyhound would recover the recessive greyhound phenotype for laxity in some dogs and the heterozygous phenotype in others. Both should be distinguishable phenotypically. The homozygotes should have low hip laxity and the heterozygotes should be lax hipped. We chose to retain those $F_1$s for backcrossing that demonstrated the highest laxity. The results of the greyhound breeding partially confirmed our approach. On the assumption that the laxity trait is dominant, the greyhound founders are probably homozygous recessive at the QTL that control hip joint laxity because the distraction indices of the nine dogs in the greyhound litter were all below the range for normal Labrador retriever distraction indices. Nevertheless, their distraction indices at 8 months of age were not all as low as the mature founding greyhounds, but their mean distraction index was not much higher than the mean distraction index of the parents. Our local environment may have had some effect on phenotypic expression of hip laxity. We do not know whether hip laxity decreases in greyhounds as they mature past 8 months of age.

Complex diseases frequently are assumed to have an additive genetic basis. Our previous crossbreedings between Labrador retrievers, golden retrievers, and beagles (Lust et al. 1973) and Gustafsson et al.'s (1975) crossbreedings between German shepherds and greyhounds indicated that the $F_1$ generation would have hip laxity, but no laxity measurements were reported. The $F_1$ progeny of German shepherds and borzois were also reported to be lax hipped (Smith G, personal communication). The tendency for the Labrador retriever–greyhound $F_1$ generation to have distraction indices similar to the Labrador retriever parent indicated that hip joint laxity may be a dominant trait but the spread of the data is wide (Figure 5). Two more breedings are planned to confirm this finding. New litters will have a female Labrador retriever parent to enable analysis of the phenotypes for their association with maternal inheritance. Establishment of the mode of inheritance of the phenotypic measurements is important because it will affect the statistical modeling for detection of linkage and the number of progeny necessary to detect linkage (Darvasi 1997).

Of interest was that the crossbred offspring of one mating of a German shepherd and greyhound by Gustafsson et al. (1975) did not have CHD, whereas 42% (8 of 19) of the German shepherd–greyhound crossbred offspring of Cardinet et al. (1983) were dysplastic. The crossbred offspring of Lust et al. (1976) from normal or dysplastic German shepherds and golden retrievers and normal beagles also had CHD in more than 50% of the dogs. German shepherds and golden retrievers may carry some alleles for CHD even if phenotypically normal and their crossbred offspring may be dysplastic. Our results are similar to those of Gustafsson et al. (1975) in the $F_1$ generation in that we had only 1 dog out of 34 that was dysplastic. Perhaps the German shepherds in Cardinet et al.'s (1983) study had more severe CHD than some of our Labrador retriever founders and the mutations are likely to be different. Combined these data suggest that the QTLs that control CHD may be expressed differently in different breeds and their crossbred offspring.

Based on our working hypothesis, that the alleles at the major QTL for laxity and the alleles at the locus "protective" against subluxation (i.e., endowing good chondro-osseous structure) are dominant, the genotype of the backcross generation could reveal how many major loci are involved in the laxity and subluxation components of the phenotype. If the backcross generation has a bimodal distribution for laxity, then one major dominant locus controls the laxity phenotype. This estimate is based on the assumption that the $F_1$ progeny is heterozygous at the loci controlling the laxity phenotype and the greyhounds are homozygous recessive (Figure 1). For two loci, the distribution will be closer to 1:3; one dog with low distraction index to three dogs with high distraction index. Similar analyses would be conducted for DLS scores. If the laxity or subluxation measurements of the backcross generation had a ratio of one tight hip or high subluxation score to three loose-hipped offspring or offspring with low subluxation scores (i.e., two major QTLs), the backcross generation would be backcrossed again to reduce the number of genes segregating that control the phenotype. This strategy is based on the idea of resolving the polygenic trait into a monogenic one and on the assumption that each QTL is inherited in a Mendelian fashion.

Inclusion of an $F_2$ generation ($F_1 \times F_1$) would enable testing the maximum number of markers for linkage to alleles segregating in the pedigree and this generation can provide an estimate of the number of QTLs controlling the phenotype based on the variances computed for the $F_1$ and $F_2$ generations (Wright 1968). The efficiency of linkage analysis for the $F_2$ generation would be optimized by including only those $F_2$s on each extreme of the laxity and dorsolateral subluxation score phenotype (Risch and Zhang 1995). The ratios of phenotypes of the $F_2$s would again verify the dominance or otherwise of the trait being analyzed. Based on distraction indices, estimates for the total number of backcross progeny to detect a QTL with a power of 50% is 242 dogs (Darvasi 1997). Two to three times as many $F_2$ progeny would be required (Darvasi 1997). Other estimates of the number of backcross or informative (at each extreme of the phenotype) $F_2$ progeny having sufficient power to detect linkage is in the range of 70–100 (Risch and Merikangas 1996; Risch and Zhang 1995). These estimates are less when based on age at onset of ossification and DLS scores because the measurements are more disparate.

Besides accurate phenotypic measurements for QTL mapping, we need molec-

ular genetic markers that are polymorphic and preferably assigned to linkage groups on a physical genetic map. Simple sequence repeats (microsatellites) are an abundant class of tandemly repeated, polymorphic DNA sequences that occur regularly throughout the genome (Ostrander et al. 1993) making them ideal genetic markers (Edwards et al. 1991; Fries et al. 1990; Holmes et al. 1993; Love et al. 1990; Ostrander et al. 1993). Based on the assumption that the canine genome is 3,000 cM long, QTL mapping studies suggest that 200–300 evenly spaced markers will span the genome with a marker spaced at an average distance of 10–20 cM (Kruglyak 1997; Lander and Schork 1994). Although there is no physical canine genetic map as yet, the first canine linkage map based on 150 canine microsatellites has been reported (Mellersh et al. 1997). The establishment of our informative outcrossed pedigree for QTL mapping of CHD will enable us to take full advantage of these canine microsatellite linkage groups as they expand.

Our data indicated that the hip phenotype may be measured by at least two components: hip joint laxity (distraction index) and chondro-osseous conformation. The latter may be measured neonatally, by the age at onset of ossification in the femoral capital chondroepiphysis and, at maturity, by the DLS score. Initial data suggested that the laxity phenotype was expressed in the backcross generation so that the outcrossed pedigree should be informative for QTL mapping of laxity, but as yet no markedly dysplastic dogs have emerged in this generation. Backcrossing to the Labrador retriever or intercrossing the $F_1$s is necessary to observe subluxation and CHD in this generation.

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